

# Considering the critical interface between tumor cells and stromal cells in the search for targets for anticancer therapy

In this issue of *Cancer Cell*, a paper by Lynch et al. demonstrates how the careful study of changes that occur at the interface between tumor cells and stromal cells led to the discovery of a new function for matrix metalloproteinase-7 (MMP-7) in the formation of osteolytic lesions in prostate cancer. The data suggest that MMP-7 inhibition could be a therapeutic target in prostate cancer.

Bone is the third most common site of metastasis in cancer patients and a major source of mortality and morbidity (Roodman, 2004). The bone is a highly organized matrix that is uniquely resistant to degradation but is the subject of constant remodeling, orchestrated by two highly specialized cells, bone-forming osteoblasts and bone-degrading osteoclasts. Through their capability to tightly bind to the bone matrix via the integrin  $\alpha\text{v}\beta3$ , osteoclasts create an extracellular phagolysosomal compartment in which the bone matrix becomes demineralized. The release into this acidic compartment of cysteine proteinases like cathepsin K and matrix metalloproteinases (MMPs) allows the degradation of collagen (Everts et al., 1992) (Blavier and Delaisse, 1995). The activity of osteoclasts is under tight control by osteoblasts. Upon hormonal stimulation by parathyroid hormone, osteoblasts become activated and express at their cell surface the receptor activator of NF $\kappa$ B ligand (RANKL), a 45 kDa glycosylated (38 kDa unglycosylated) protein with a transmembrane domain

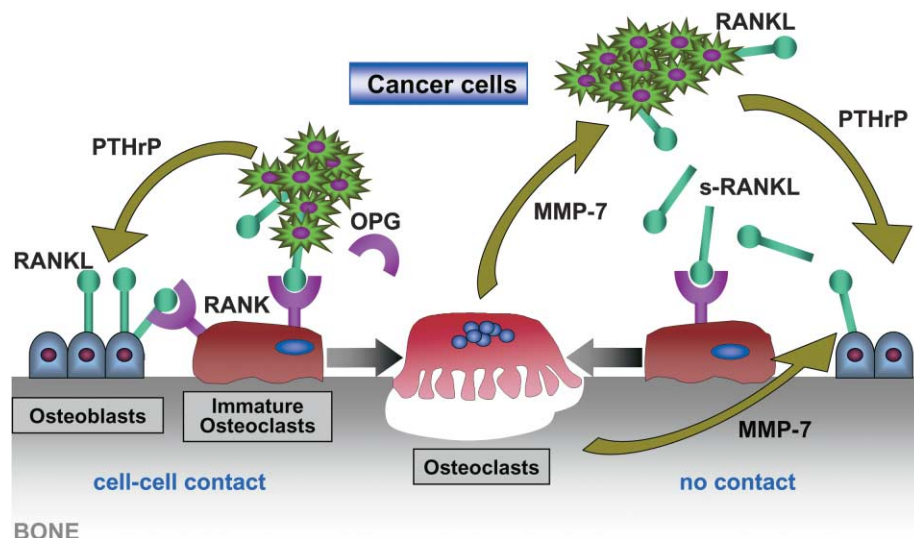
(Takayanagi et al., 2002). Close contact between osteoblasts and osteoclast precursor cells promotes RANKL binding to its receptor RANK and osteoclast differentiation and activation. RANKL activation of RANK is further regulated by osteoprotegerin (OPG), a soluble RANKL decoy receptor that interferes with RANK-RANKL interaction. Tumor cells do not have the capability to resorb bone, but through the expression of the osteoblast activator parathyroid hormone related peptide (PTHrP) and RANKL can activate osteoclasts (Mundy, 2002) (Figure 1).

In this issue, the article by Lynch et al. (2005) illustrates how the careful study of changes that occur in the bone microenvironment can lead to findings of importance in our search for novel targets for therapeutic intervention in cancer. These investigators have developed a new in vivo model in which prostatic carcinoma cells obtained in rats treated with the carcinogen DMAB (3-2' dimethyl 4-aminobiphenyl) and testosterone propionate are transplanted into the cranial region of syngeneic rats or immunodeficient

(RAG-2 deficient) mice. After 2 to 4 weeks, they observed radiological and histopathological changes in the calvaria that closely reproduce osteolytic and osteoblastic alterations seen in human prostate cancer bone metastasis. Using a combination of RT-PCR and microarray gene expression analysis on material specifically collected at the tumor-bone interface, they documented, not surprisingly, elevated levels of PTHrP, RANKL, and cathepsin K, and decreased levels of OPG over time (although OPG levels were elevated at the beginning). Interestingly, they also observed elevated levels of MMP-7, a member of the MMP family of proteases that has been previously reported to be overexpressed in epithelial cancers, including breast and prostate. This was an unexpected observation. Many MMPs (9, 10, 12, and 14) have been shown to be expressed by osteoclasts, but not MMP-7 (Delaisse et al., 2003). Furthermore, the finding that MMP-7 was expressed by osteoclasts and not by prostate cancer cells was at odds with previous observations demon-

**Figure 1.** MMP-7 and osteoclast activation in cancer

Membrane-associated RANKL expression by tumor cells or by PTHrP stimulated osteoblasts promotes the maturation and activation of osteoclast precursor cells through close cell-cell contact. Cleavage and solubilization of RANKL by MMP-7 allows the stimulation of osteoclasts without the need for cell-cell contact.



strating that MMP-7 in tumors is primarily expressed by malignant cells. A clue, however, came from a previous observation made by the laboratory of Dr. Matrisian, who contributed to the paper. Her laboratory had demonstrated that MMP-7 can cleave substrates other than extracellular matrix (ECM) proteins, and in particular TNF- $\alpha$ , mediating therefore cell-cell communication rather than matrix degradation (Haro et al., 2000). Aware that RANKL is a member of the TNF- $\alpha$  family of ligands, the authors postulated and then elegantly demonstrated that MMP-7 cleaves RANKL at residues 145–146 in the stalk region of the protein, releasing an active soluble form of RANKL from the cell surface. The solubilization of RANKL is not without significant consequence, because it eliminates the need for close contact between RANKL-expressing cells like osteoblasts and tumor cells and RANK-expressing osteoclast precursor cells. That MMP-7 is essential for bone resorption in their model was then confirmed in immunodeficient mice in which MMP-7 had been knocked down.

The paper illustrates several important aspects in our efforts to identify new targets for therapeutic strategies. In searching for such targets, it is important to consider the stromal components of a tumor (the microenvironment). Well aware of this, the investigators purposely performed their gene expression analysis on material obtained at the tumor-bone interface. However, because they used a syngeneic model, it was not possible by gene array analysis only to identify the source of expression of the genes they found to be up- or downregulated. They had to perform a careful immunohistological analysis to discover that, in an unanticipated manner, MMP-7 was expressed not by prostate cancer cells, but rather by osteoclasts. Human xenotransplanted models in mice, used by many laboratories, have a significant advantage in this aspect, because they allow differentiation of the source of the genes expressed on the basis of the species (human versus murine). The development of gene arrays in which

human and murine sequences are compared, such as that recently developed by the Protease Consortium (Hu/Mu ProtIn, Affymetrix), will allow us to address the critical question of the contribution of the host microenvironment in these models. A second point that the paper illustrates is that dogma, although helpful, should always be revisited. In the 1980s and early 1990s, the consensus was that the proteolytic activity of MMPs was primarily directed toward ECM proteins (as reflected by their designation as matrix proteases). In the late 1990s and early 2000s, it became apparent that MMPs can proteolytically process a large number of growth factors, growth factor receptors, and cytokines, and affect their solubilization and biological activity (McCawley and Matrisian, 2001). Another dogma is that in contrast to most MMPs, which are expressed by tumor cells and stromal cells, MMP-7 was considered to be primarily expressed by tumor cells. The authors demonstrate that this is clearly not always the case. An important last lesson that this paper illustrates is that biomedical research, although moving from the laboratory to the bedside, needs also to be able to return from the bedside to the laboratory. On the basis of promising preclinical studies, most of them performed in experimental metastatic models, synthetic MMP inhibitors were tested in clinical trials many years ago. However, the results of these clinical trials were disappointing, and in some cases indicated accelerated progression (Coussens et al., 2002). As a result, most trials with MMP inhibitors have been abandoned, and the use of MMP inhibitors as anti-cancer agents has been considered a failure. Lynch et al. now suggest that there could be a very specific window in cancer progression where the use of a specific MMP-7 inhibitor in addition to other therapies could inhibit bone invasion by prostate cancer cells. Patients with prostate cancer and bone metastases were not included in previous clinical trials. It is not unusual to see therapeutic agents developed in the past and abandoned because of a lack of

apparent effect in clinical trials returning later to the clinic after a better understanding of their biological activity. To convince the pharmaceutical industry to continue to make MMP inhibitors available for laboratory research will be critical in our effort to revisit the use of such agents in cancer therapy.

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